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Abstract

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Project Title:	Targeting a Protein Interaction Site on Smad Transcription Factors

Abstract: *DESCRIPTION (provided by applicant):* A high throughput screening assay will be provided to screen the MLSCN library for compounds that disrupt a key protein-protein interaction site on the Smad transcription factors that mediate cellular responses to transforming growth factor beta (TGF-a). There are currently no small molecule ligands that target Smad proteins. Inhibition of Smad function will be an important research tool in elucidating the multiple biological functions of TGF-a. Inhibition of TGF-a signaling is also a validated target in several advanced cancers because of its role in facilitating cancer cell migration, proliferation, metastasis to bone and/or evasion of the immune response. It is also a validated target in all forms of fibrotic disease including idiopathic pulmonary fibrosis and diabetic nephropathy. Current strategies for inhibiting TGF-a signaling focus on disruption of the ligand-receptor interaction with neutralizing antibodies or inhibition of the TGF-a receptors using small molecule inhibitors of the kinase activity. This proposal targets the transcriptionally active Smad complexes to inhibit signaling. The working hypothesis is that disruption of only one binding site on Smad will block binding of some but not all Smad binding partners (over two dozen different transcription factors, coactivators and co-repressors), thereby interfering with only a subset of the gene expression responses mediated by Smad complexes. One advantage of this strategy over inhibiting ligand binding or receptor kinase activity is that it might provide selective inhibition of some but not all TGF-a responses. The assay is a homogeneous time resolved fluorescence resonance transfer assay that measures peptide binding to a specific protein binding site in the hydrophobic corridor of Smad2 and Smad3. The binding site is present only in Smad2 and Smad3 and not in Smads 1, 4, 5, 6, 7, or 8, therefore, ligands to this site should have preferential effects on Smad2 and Smad3 functions. Furthermore, missense mutations in the binding site indicate that the ligands for the site, the FoxH1 SIM, the SARA rigid coil and the nuclear porin FG repeat, contact overlapping but not identical amino acids, suggesting that a small molecule ligand might preferentially block binding of one of these proteins to Smad2 or Smad3. The assay has a Z' factor of 0.7- 0.8 in a 384-well plate format and has been used to screen 16,000 compounds at the Keck-UWCCC Small Molecule Screening Facility. This preliminary screen led to the identification of two compounds that in secondary assays

also inhibit TGF-a stimulation of reporter gene expression in HepG2 cells. If the aims of this application are achieved, new compounds targeted to the Smad protein hydrophobic corridor would become critical research reagents to demonstrate which normal or pathological responses to TGF-a depend on this Smad protein binding site. Given the need for new therapeutic approaches in diseases where TGF-a signaling is an important target, such as glioma and idiopathic pulmonary fibrosis, active compounds identified by the proposed assay would stimulate additional pharmaceutical development and clinical trials.

Thesaurus Terms: high-throughput screening, HTS, Molecular Libraries Screening Centers Network, MLSCN, protein-protein interaction, Smad transcription factors, transforming growth factor beta, TGF-a, cancer cell migration, pulmonary fibrosis, fibrotic disease, diabetic nephropathy, small molecule inhibitors, fluorescence resonance transfer assay, peptide binding, hydrophobic corridor, Smads 1, Smad2, Smad3, Smad 4, Smad 5, Smad 6, Smad 7, Smad 8, FoxH1 SIM, SARA rigid coil, nuclear porin FG, 384-well plate format, HepG2 cells, glioma

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